Scientific Bulletin. Series F. Biotechnologies, Vol. XXII, 2018
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

COLD/HEAT SHOCK PRE-TREATMENTS FOR GYNOGENIC HAPLOID EMBRYO INDUCTION IN *Amaryllıdaceae* AND *Cucurbıtaceae*: A REVIEW

Faika YARALI KARAKAN, Bekir Bülent ARPACI

Kilis 7 Aralik University, Faculty of Agriculture, Department of Horticulture, Kilis, Turkey

Corresponding author email: faikayarali@gmail.com

Abstract

Pre-treatments significantly affect frequency of gynogenic embryogenesis. In gynogenesis, a change from gametophytic phase to the sporophytic phase is provided by stress treatments. Heat and/or cold shocks are given alone or in combination with each other to induce stress conditions. Also, pre-treatments can be applied at different types of explant, such as flower buds, ovules/ovaries, or inflorescence. With regard to different explants, the type, level, and duration of pre-treatment are different, and the regeneration efficiencies vary as well. In this review, it was discussed the effects of pre-treatments, cold or heat shock, in haploid induction via gynogenesis in different vegetables species.

Key words*: Amaryllidaceae, Cucurbitaceae, Gynogenesis, pre-treatments.*

INTRODUCTION

Plants with gametophytic chromosome number in their sporophyte whether diploid or polyploid chromosome number are named haploids (Chen et al., 2011). *In vitro* haploid production is very important in plant breeding because of the shortening of the time required for the production of homozygous lines compared with conventional breeding methods (Shalaby, 2007; Chen et al., 2011). Haploid plants originating from gametes carry the genetic information of only one set of chromosomes, so they can be regarded as genetically homozygotic. Male and female gametophytes are used in production of haploid plants. The sporophytic developmental pathway starting from immature female gametes is known as *in vitro* gynogenesis (Juhasz and Jakse, 2005; Yarali and Yanmaz, 2013). *In vitro* haploid production via gynogenesis has been routinely used for inbred production in many *Amaryllidaceae* and *Cucurbitaceae* species (Gupta and Singh, 2016; Yarali and Yanmaz, 2017). Gynogenesis consists of *in vitro* culture of unfertilized ovule, ovary and whole flower buds (Asif, 2013; Yarali and Yanmaz, 2013; Gupta and Singh, 2016). One of the most important points for success in ovule, ovary or flower bud cultures is the size of the flower bud, that is, the period of ovule development (Mukhambetzhanov, 1997; Bohanec, 2009). However, due to the lack of information on what is the molecular mechanism that triggers gynogenetic development, it is not known exactly how the gynogenesis phenomenon occurs at the cellular level. Many researchers have been recommended that female gametes of flower buds used gynogenesis studies should be collected before anthesis (Bohanec, 2009; Palmer and Keller, 2005; Chen et al., 2011; Asif, 2013). Asif (2013) expressed that development stage of microspores is an excellent indicator to identify the exact time for *in vitro* culture. The most responsive ovaries/ovules had nearly mature or fully mature embryo sacs (Gemes-Juhasz et al., 2002).

The most common factor affecting embryogenesis is stress treatments. Cold or heat shock are commonly used for induce stress conditions. The floral organs are pre-treated to stimulate the process of sporophytic development in female gametophytes. The essential benefits of pre-treatment for stock plants were to eliminate variation arising from outer factors. Without imposing stress, a change from gametophytic to the sporophytic phase is very difficult. Pre-treatments can be applied at different types of explant, such as flower buds, isolated ovules/ovaries, or inflorescence. With regard to different explants, the type, level, and duration of pre-treatment are different, and the regeneration efficiencies

vary as well. However, duration, time, type, and level of pre-treatment vary considerably from one species to another (Asif, 2013; Chen et al., 2011; Dhatt and Thakur, 2014). As a result of these stress factors, leading to the formation of gynogenetic embryos or morphogenic callus, the development of the gametes is diverted from the gametophytic path of development to the sporophytic path (Keller and Korzun, 1996; Chen et al., 2011). However, there are not enough researches on the effect of pre-treatments on embryo induction *in vitro* gynogenesis. In this review, it was discussed the effects cold or heat shock pre-treatments in different vegetables species in *Amaryllidaceae* and *Cucurbitaceae* that gynogenesis has been the most successful method used for haploid plants production.

***Amaryllidaceae* - species:**

The family *Amaryllidaceae* has a large number of species such as onion, garlic and leek. Doubled haploids were obtained successfully from these species using dihaploidization techniques (Yaralı and Yanmaz, 2016). However, more research has been conducted out in onion (*Allium cepa* L.) via gynogenesis. For this reason, researchs on onion are mainly evaluated in this part.

Puddephat et al. (1999) investigated that the effect of temperature pre-treatments of stock ovaries on gynogenic embryogenesis induction. Flower buds of onion excised from stock plants maintained at 15ºC with natural daylight to flower under glasshouse conditions were ten times more responsive than those taken from plants raised under glasshouse conditions, or held at 10ºC. In addition, they found that decreasing donor plant growth temperature in the final phases of flower development increased the efficiency of gynogenesis. Similarly, Alan et al. (2004) reported that flower buds from onion plants stored at 10ºC for 4-23 days in beakers of water had more responsive to induction of gynogenesis and were comparable to fresh flower-buds. In another study, Hanna (1994), reported that cold pre-treatment at 4ºC for 4 days enhanced gynogenic embryo induction in onion. In contrast to these findings, Keller and Korzun (1996) stated that the 30°C temperature pre-treatment was suppressed embryo regeneration in onion. Cold treatments either had no effect or had negative effects in gynogenic embryo induction. In another study used different *Allium* species and varieties, was found that the low temperature applied to flower buds before culture was particularly inhibiting effect on the leek, and there was no effect on the hybrid cultivars (Keller, 1990). Schum et al. (1993) used a dark preculture at 10°C for up to 12 days but did not obtain consistent results for all genotypes. A heat pre-treatment led to suppression of regeneration. No or even negative effects of cold pre-treatment were reported by Muren (1989) (Keller and Korzun, 1996). Hassandokht and Campion (2002), investigated the effect of cold treatment at 17°C applied to flowers in culture was evaluated in six Iranian and two Italian onions. The results showed that an inhibitory effect of cold on haploid formation.

***Cucurbitaceae* - species:**

Diao et al. (2009) investigated the effect of thermal shock pre-treatment at 35^{o}C for different period of time on embryo formation via gynogenesis (ovary culture) in *Cucumis sativus* L. The results showed that heat shock treatment for 3 days at 35^{o}C at the beginning of the culture had higher embryo formation rate than 2 or 4 days. Gemes-Juhasz et al. (2002), aimed produced gynogenic plants of pickling cucumber via gynogenesis. They extracted cucumber ovaries and placed on induction media and cultured in the dark conditions for 2–5 days, at 24^{o}C, 28°C or 35^{o}C. They found that the heat treatment increased the efficiency of gynogenesis. The highest number of embryos occurred following the 35^{o}C induction treatment. The flow cytometry analysis showed that 87.7% of the regenerants were haploid. In another study by conducted out by Wang et al. (2008) was stated that 36°C pre-treatment could induced high frequency embryoids than the 4ºC pre-treatment in cucumber. Shalaby, (2007) investigated that influence of temperature (4ºC and 32°C) for 0, 4, 7 and 12 days on the ovule culture the *in vitro* gynogenesis induction of squash. According to research datas, ovules incubated at 4 or 32°C for 4 days produced a better embryogenic response than others treatments. Contrary to positive results in hot shock temperature

treatments, some researchers suggested that cold treatments were more efficient for induction of gynogenic embryogenesis. For example, Kwack and Fujieda (1988), found that the cold treatment of *Cucurbita moschata* ovaries at 5°C for 2 days was efficient for embryogenesis.

Despite the cited examples of a positive influence of cold or heat treatment, Bohanec (1998) suggested that *in vitro* gynogenesis is generally not stimulated by shock treatment such as low or high temperatures. For example, in *Cucurbita pepo* Metwally et al. (1998), found no beneficial effect of cold pre-treatment on gynogenesis. They picked ovaries from squash plants and exposed to cold temperature (4°C) for 0, 2, 4 and 8 days and ovules were cultured. Then the dishes were incubated at 25 ± 1°C under 16 h photoperiod for 4 weeks. Data from the research indicated that cold treatment at 4°C for 2, 4 or 8 days suppressed of embryogenesis compared with the control. The control ovules gave the highest embryogenic ovules per 100 cultured ovules. Similarly, Yang and Zhou (1982) reported that cold temperature pre-treatment was ineffective in ovary and ovule culture of most species.

CONCLUSIONS

In this review, it was evaluated the effects of cold or heat shock pre-treatments in different vegetables species in *Amaryllidaceae* and *Cucurbitaceae* that gynogenesis has been the most successful method used for haploid plants production. When the researches are evaluated, it can be said that cold and hot pre-treatments have positive effects on the haploid embryo induction. But the numbers of researches on pre-treatments are not enough. Therefore, more research is needed to make a clearer determination about effects of temperature pre-treatments.

REFERENCES

Alan A.R., Brants A., Cobb E., Goldschmied A., Mutschler M.A., Earle E.D., 2004. Fecund gynogenic lines from onion (*Allium cepa* L.) breeding materials. Plant Science, Volume: 167(5): 1055-1066.

Asif M., 2013. Progress and opportunities of doubled haploid production. Springer Briefs in Plant Science, Springer International Publishing Switzerland.

Bohanec B., 1998. Gynogenic haploid induction in plant breeding. In: Hansen M (ed) COST 824, gametic embryogenesis, report of activities 1995–1997. European Commission, Directorate General Science, Research, Development, Luxembourg, pp 253–255.

Bohanec B., 2009. Doubled haploids via gynogenesis. advances in haploid production in higher plants. Ed.: Touraev vd., Springer Science Business Media B.V., Page: 35-46.

Chen J.F., Cui L., Malik A.A., Mbira K.G., 2011. *In vitro* haploid and dihaploid production via unfertilized ovule culture. Plant Cell Tiss. Organ Cult., Volume: 104, Pages: 311-319. DOI 10.1007/s11240-010-9874-6.

Dhatt A.S., Thakur P., 2014. Production of doubled haploids in onion: A review. J. Hort. Sci. Vol: 9(2): 107-112.

Diao W.P., Jia Y.Y., Song H., Zhang X.Q., Lou Q.F., Chen J.F., 2009. Efficient embryo induction in cucumber ovary culture and homozygous identification of the regenetants using SSR markers. Sci Hortic 119:246–251.

Gemes-Juhasz A., Balogh P., Ferenczy A., Kristo´f Z., 2002. Effect of optimal stage of female gametophyte and heat treatment on *in vitro* gynogenesis induction in cucumber (*Cucumis sativus* L.). Plant Cell Rep 21:105–111

Gupta A.J., Singh B.K., 2016. Development of Hybrids and Hybrid Seed Production of Onion. Training Manual on „Principles and Production Techniques of Hybrid Seeds in Vegetables", p:101-110.

Hanna A.B., 1994. Genetic evaluation of some economic characters in onion (*Allium cepa*). Ph.D. Thesis, Fac. of Agric. Moshtohor, Zagazig Univ. Banha branch.

Hassandokht M.R, Campion B., 2002. Low temperature, medium and genotype effect on the gynogenic ability of onion (*Allium cepa* L.) flowers cultured in vitro. Advances in Horticultural Science, 16(2): 1-7.

Juhász A.G., Jakše M., 2005. Haploids in the improvement of miscellaneous crop species (*Cucurbitaceae, Liliaceae, Aspragaceae, Chenopediaceae, Araceae* and *Umbelliferae*). In: Palmer C.E., Keller W.A., Kasha K.J., editors. Biotechnology in Agriculture and Forestry, Vol. 56: Haploids in Crop Improvement II. Berlin, Germany: Springer–Verlag, pp. 259–276.

Keller J., 1990. Results of anther and ovule culture in some species and hybrids in the genus *Allium* L. Archiv für Züchtungsforschung, vol: 20, no:3, page: 189-197.

Keller E.R.J., Korzun L., 1996. Haploidy in onion (*Allium cepa* L.) and other *Allium* species. In: Jain, S.M., Sopory, S.M. and Veilleux, R.E. (eds) In Vitro Haploid Production in higher plants, Vol: 3 Important Selected Plants. Kluwer Academic Publisher, Netherlands.

Kwack S.N., Fujieda K., 1988. Somatic embryogenesis in cultured unfertilized ovules of *Cucurbita moschata*. J. Jpn. Soc. Hort. Sci. 57: 34–42.

Metwally E., Moustafa S.A., El-Sawy B.I., Harun S.A., Shalaby T.A., 1998. Production of haploid plants from *in vitro* culture of unpollinated ovules of *Cucurbita pepo*. Plant Cell Tissue Organ Cult 52:117–121

Mukhambetzhanov S.K., 1997. Culture of Nonfertilized Female Gametophytes *in vitro*. Plant Cell, Tissue and Organ Culture, 48: 111-119.

Muren R., 1989. Haploid plant induction from unpollinated ovaries in onion. HortScience 24(5): 833- 834.

Palmer C.E.D., Keller W.A., 2005. Overview of haploidy. biotecnology in agriculture and forestry, haploids in crop imprevement II (Ed. By Palmer CE, Keller WA and Kasha KH), Vol:56, pp:3-7

Puddephat I.J., Robinson H.T., Smith B.M., Lynn J., 1999. Influence of stock plant pre-treatment on gynogenic embryo induction from flower buds of onion. Plant Cell, Tissue and Organ Culture. 57: 145-148.

Schum A., Mattiesch L., Timmann E.M., Hofmann K., 1993. Regeneration of dihaploids via gynogenesis in *Allium porrum* L. Gartenbauwissenschaft, 58:227–232

Shalaby T., 2007. Factors affecting haploid induction through *in vitro* gynogenesis in summer squash (*Cucurbita pepo* L.), Scientia Horticulturae 115(115):1-6.

Wang Y., Gu X., Zhaing S., 2008. Study the effect of pre-treatment and exogenous hormones on embryoids induced from unpollinated ovaries of cucumber. Acta Agriculturae Boreali-Sinica, 2008, 23(S1): 50-53.doi: 10.7668/hbnxb.2008.S1.013.

Yang H.Y., Zhou C., 1982. *In vitro* induction of haploid plants from unpollinated ovaries and ovules. Theor Appl Genet 63:97–10

Yarali F., Yanmaz R., 2013. Utilization of haploidy techniques in breeding of *Allium* species. Turk. J. Sci. Rev. 6(2):44-50.

Yarali F., Yanmaz R., 2016. The effects of nedia composition on *in vitro* gynogenic embryo induction in *Allium tuncelianum* (Kollman) Özhatay, Matthew, Şiraneci). International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 8(58): 3247-3251.

Yarali F., Yanmaz R., 2017. The effects of plant growth regulators on *in vitro* gynogenic embryo formation in onion (*Allium cepa* L.). Afr. J. Biotechnol, 16(40): 1977-1983.